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NES-1 POLYPEPTIDES, DNA, AND RELATED MOLECULES AND METHODS
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REPLACEMENT SHEET

76N 76R-30

28\$[

185[



FIG. 1



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Vimla Band 10/021,368 00398-100005 REPLACEMENT SHEET

MDA-468(BT) MDA-436(BT 39VTE7(NEI) 21MT-1(BT 76R30(RT) T47-D(BT 7VNF(NF) 4VNF(NF) 76N(NE) 4VN(NE) 3VN(NE) FS-2(F) NES1

> M2E6E7(MI) M3E6E7(MI)

76NF(NF) **6VNF(NF)** 

3VN(NE)

NES<sub>1</sub>

7VNE6(NI)

MDA-231(BT) MDA-435(BT)

ZR-75-1(BT) HBL-100(MI) HS578T(BT)

MDA-157(BT) MCF-10A(NI)

MCF-7(BT)

Caski(CT) Hela(CT) Siha(CT)



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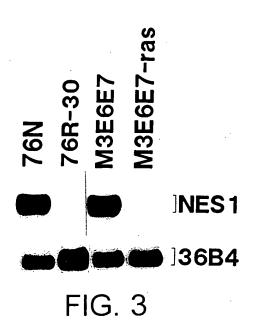


FIG. 4



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NES-I POLYPEPTIDES, DNA, AND RELATED MOLECULES AND METHODS
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## **Histone 3.2** 

● ● ● ● 136B4

FIG. 5A

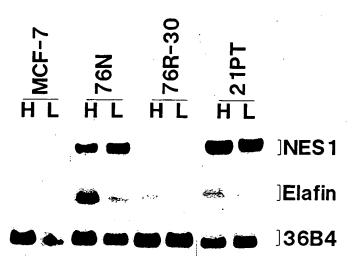
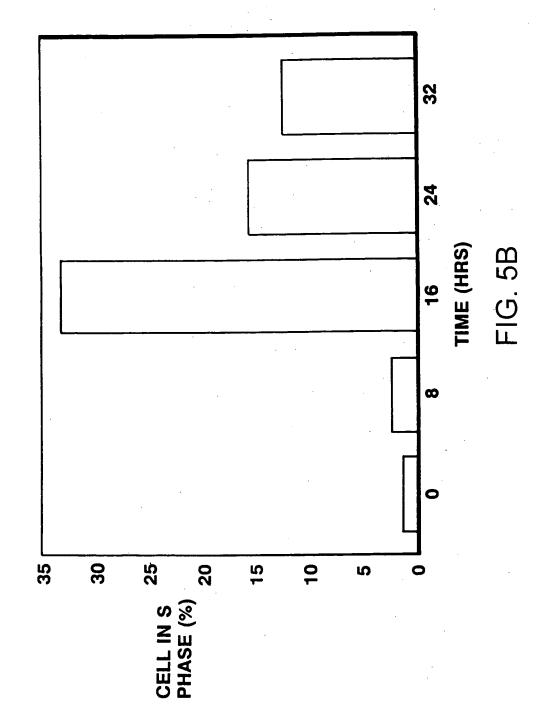


FIG. 6



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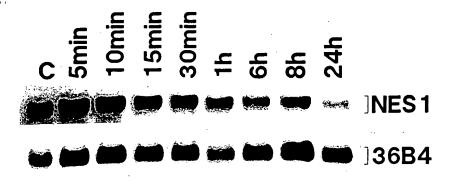


FIG. 7A



c PMA 1 2 NES1 36B4 FIG. 8



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### Comparison of Sequences near putative active site

								•		
	9	137	223	227	228	230	231	241	243	245
NES 1	H	D	D P	S	D S	G	G	G	Ļ	W
Human Pancreatic	Н	D	D S	R	D S	G	G	G	٧	W
Trypsinogen III	<b>A</b>	<b>A</b>	ΔΔ		<b>A</b>			Δ	Δ	Δ

A: Catalytic triad

 $\triangle$ : Residues important for substrate binding and specificity

FIG. 9A

### Alignment of NES1 amino acid sequence with other serine proteases

	1			,	50
Mmtryar	MSALLILA	LVGA		AVAFPVDD.D	DKIVGGY
Hstryivb		LLGGRTWRAA	RDADGCEALG	TVAVPFDD.D	DKIVGGY
Rntrypvb	MKICIFFT	LLGT		VAAFPTEDND	DRIVGGY
Sstrypii				.AAFATED	DKIVGGY
Nesl	MRAPHLHLSA	ASGARALAKL	LPLLMAQLWA	AEAALLPQND	TRLDPEAYGA
				-	
	51				100
Mmtryar	TCRESSVPYQ	VSLNAGYHF.	CGGSLINDQW	VVSAAHCYKY	RIQVRLGEHN
Hstryivb	TC.ENSLPYQ	VSLNSGSHF.	CGGSLISEQW	VVSAAHCYKT	RIQVRLGEHN
Rntrypvb	TCQEHSVPYQ	VSLNAGSHI.	CGGSLITDQW	VLSAAHCYHP	QLQVRLGEHN
Sstrypii	ECKAYSQPHQ	VSLNSGYHF.	CGGSLVNENW	VVSAAHCYQS	RVEVRLGEHN
Nesl	PCARGSQPWQ	VSLFNGLSFH	CAGVLVDQSW	VLTAAHCGNK	PLWARVGDDH
					<b></b> ·
•	101	•			150
Mmtryar	INVLEGNEQF	VDSAKIIRHP	NYN	.SWTLDNDIM	LIKLASPVTL
Hstryivb	IKVLEGNEQF	INAAKIIRHP	KYN	.RDTLDNDIM	LIKLSSPAVI
Rntrypvb	IYEIEGAEQF	IDAAKMILHP	DYD	.KWTVDNDIM	LIKLKSPATL
Sstrypii	IQVTEGSEQF	ISSSRVIRHP	NYS	.SYNIDNDIM	LIKLSKPATL
Nesl	LLLLQG.EQL	RRTTRSVVHP	KYHQGSGPIL	PRRTDEHDLM	LLKLARPVVP
		_	<u> </u>		
			EYTDA INISI	<del></del>	
	151		EXTRA INSE	AT -	200
Mmtryar	151 NARVASVPLP	SSCAPAGTQC		NGVNNPDLLQ	
Mmtryar Hstryivb		SSCAPAGTQC TAPPAAGTEC	LISGWGNTLS		CVDAPVLPQA
-	NARVASVPLP		LISGWGNTLS LISGWGNTLS	NGVNNPDLLQ FGADYPDELK	CVDAPVLPQA CLDAPVLTQA
Hstryivb	NARVASVPLP NARVSTISLP	TAPPAAGTEC	LISGWGNTLS LISGWGNTLS	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ	CVDAPVLPQA CLDAPVLTQA
Hstryivb Rntrypvb	NARVASVPLP NARVSTISLP NSKVSTIPLP	TAPPAAGTEC QYCPTAGTEC	LISGWGNTLS LISGWGNTLS LVSGWG.VLK	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD.KNKLQ	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS
Hstryivb Rntrypvb Sstrypii	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD.KNKLQ	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS
Hstryivb Rntrypvb Sstrypii	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD.KNKLQ	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS
Hstryivb Rntrypvb Sstrypii	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK
Hstryivb Rntrypvb Sstrypii Nesl	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA EGGKDSCQGD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD.KNKLQ RRVKYNKGLT SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK 250
Hstryivb Rntrypvb Sstrypii Nes1	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201 DCEASYPGDI	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA EGGKDSCQGD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK  250 LQGIVSWG.Y LQGVVSWG.H
Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201 DCEASYPGDI ECKASYPGKI	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL TNSMFCVGFL	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA  EGGKDSCQGD EGGKDSCQGD EGGKDSCQRD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD.KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK  250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D
Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb Rntrypvb	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP  201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL TNSMFCVGFL TNNMFCLGFL	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA  EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGQ SGGPVVCNGQ	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK  250 LQGIVSWG.Y LQGVVSWG.H
Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb Rntrypvb Sstrypii	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP  201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI DCNNSYPGMI	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL TNSMFCVGFL TNNMFCLGFL TNAMFCAGYL	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA  EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK  250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y
Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb Rntrypvb Sstrypii	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP  201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI DCNNSYPGMI	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL TNSMFCVGFL TNNMFCLGFL TNAMFCAGYL	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA  EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD DRGQDPCQSD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK  250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y LQGILSWGVY
Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb Rntrypvb Sstrypii	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP  201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI DCNNSYPGMI ECEVFYPGVV	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC  TNNMICVGFL TNSMFCVGFL TNNMFCLGFL TNAMFCAGYL TNNMICAG.L	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA  EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD DRGQDPCQSD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK  250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y LQGILSWGVY
Hstryivb Rntrypvb Sstrypii Nes1  Mmtryar Hstryivb Rntrypvb Sstrypii Nes1	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP  201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI DCNNSYPGMI ECEVFYPGVV  251	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC  TNNMICVGFL TNSMFCVGFL TNNMFCLGFL TNAMFCAGYL TNNMICAG.L	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA  EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD DRGQDPCQSD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK  250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y LQGILSWGVY
Hstryivb Rntrypvb Sstrypii Nes1  Mmtryar Hstryivb Rntrypvb Sstrypii Nes1	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP  201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI DCNNSYPGMI ECEVFYPGVV  251 GCAQPDAPGV	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC  TNNMICVGFL TNSMFCVGFL TNNMFCLGFL TNAMFCAGYL TNNMICAG.L  YTKVCNYVDW	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA  EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD DRGQDPCQSD  280 IQNTIADN*.	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK  250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y LQGILSWGVY
Mmtryar Hstryivb Rntrypvb Sstrypii Nes1  Mmtryar Hstryivb Rntrypvb Sstrypii Nes1  Mmtryar Hstryivb	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP  201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI DCNNSYPGMI ECEVFYPGVV  251 GCAQPDAPGV GCAWKNRPGV	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC  TNNMICVGFL TNSMFCVGFL TNMFCLGFL TNMMCAGLL YTKVCNYVDW YTKVCNYVDW	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA  EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD DRGQDPCQSD  280 IQNTIADN*. IKDTIAANS*	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK  250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y LQGILSWGVY
Mmtryar Hstryivb Rntrypvb Sstrypii Nes1  Mmtryar Hstryivb Rntrypvb Sstrypii Nes1  Mmtryar Hstryivb Rntrypvb	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP  201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI DCNNSYPGMI ECEVFYPGVV  251 GCAQPDAPGV GCAWKNRPGV GCALEGKPGV	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC  TNNMICVGFL TNSMFCVGFL TNMFCLGFL TNMFCAGYL TNMMICAG.L  YTKVCNYVDW YTKVYNYVDW YTKVYNYVDW YTKVCNYLNW YAKVCIFNDW	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA  EGGKDSCQGD EGGKDSCQRD EGGKDSCQRD EGGKDSCQRD DRGQDPCQSD  280 IQNTIADN*. IKDTIAANS* IQQTVAAN*.	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK  250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y LQGILSWGVY



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1 MRAPHLHLSA ASGARALAKL LPLLMAQLWA AEAALLPQND TRLDPEAYGA
51 PCARGSQPWQ VSLFNGLSFH CAGVLVDQSW VLTAAHCGNK PLWARVGDDH
101 LLLLQGEQLR RTTRSVVHPK YHQGSGPILP RRTDEHDLML LKLARPVVPG
151 PRVRALQLPY RCAQPGDQCQ VAGWGTTAAR RVKYNKGLTC SSITILSPKE
201 CEVFYPGVVT NNMICAGLDR GQDPCQSDSG GPLVCDETLQ GILSWGVYPC
251 GSAQHPAVYT QICKYMSWIN KVIRSN\* (SEQ ID NO: 1)

FIG. 10

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TRADER!	ACCAGCGGCA	GACCACAGGC	AGGGCAGAGG	CACGTCTGGG	TCCCCTCCCT
51	CCTTCCTATC	GGCGACTCCC	AGATCCTGGC	CATGAGAGCT	CCGCACCTCC
101	ACCTCTCCGC	CGCCTCTGGC	GCCCGGGCTC	TGGCGAAGCT	GCTGCCGCTG
151	CTGATGGCGC	AACTCTGGGC	CGCAGAGGCG	GCGCTGCTCC	CCCAAAACGA
201	CACGCGCTTG	GACCCCGAAG	CCTATGGCGC	CCCGTGCGCG	CGCGGCTCGC
251	AGCCCTGGCA	GGTCTCGCTC	TTCAACGGCC	TCTCGTTCCA	CTGCGCGGGT
301	GTCCTGGTGG	ACCAGAGTTG	GGTGCTGACG	GCCGCGCACT	GCGGAAACAA
351	GCCACTGTGG	GCTCGAGTAG	GGGATGATCA	CCTGCTGCTT	CTTCAGGGCG
401	AGCAGCTCCG	CCGGACGACT	CGCTCTGTTG	TCCATCCCAA	GTACCACCAG
451	GGCTCAGGCC	CCATCCTGCC	AAGGCGAACG	GATGAGCACG	ATCTCATGTT
501	GCTAAAGCTG	GCCAGGCCCG	TAGTGCCGGG	GCCCGCGTC	CGGGCCCTGC
551	AGCTTCCCTA	CCGCTGTGCT	CAGCCCGGAG	ACCAGTGCCA	GGTTGCTGGC
601	TGGGGCACCA	CGGCCGCCCG	GAGAGTGAAG	TACAACAAGG	GCCTGACCTG
651	CTCCAGCATC	ACTATCCTGA	GCCCTAAAGA	GTGTGAGGTC	TTCTACCCTG
701	GCGTGGTCAC	CAACAACATG	ATATGTGCTG	GACTGGACCG	GGGCCAGGAC
751	CCTTGCCAGA	GTGACTCTGG	AGGCCCCCTG	GTCTGTGACG	AGACCCTCCA
801	AGGCATCCTC	TCGTGGGGTG	TTTACCCCTG	TGGCTCTGCC	CAGCATCCAG
851	CTGTCTACAC	CCAGATCTGC	AAATACATGT	CCTGGATCAA	TAAAGTCATA
901	CGCTCCAACT	GATCCAGATG	CTACGCTCCA	GCTGATCCAG	ATGTTATGCT
951	CCTGCTGATC	CAGATGCCCA	GAGGCTCCAT	CGTCCATCCT	CTTCCTCCCC
1001	AGTCGGCTGA	ACTCTCCCCT	TGTCTGCACT	GTTCAAACCT	CTGCCGCCCT
1051	CCACACCTCT	AAACATCTCC	CCTCTCACCT	CATTCCCCCA	CCTATCCCCA
1101	TTCTCTGCCT	GTACTGAAGC	TGAAATGCAG	GAAGTGGTGG	CAAAGGTTTA
1151	TTCCAGAGAA	GCCAGGAAGC	CGGTCATCAC	CCAGCCTCTG	AGAGCAGTTA
1201	CTGGGGTCAC	CCAACCTGAC	TTCCTCTGCC	ACTCCCCGCT	GTGTGACTTT
1251	GGGCAAGCCA	AGTGCCCTCT	CTGAACCTCA	GTTTCCTCAT	CTGCAAAATG
1301	GGAACAATGA	CGTGCCTACC	TCTTAGACAT	GTTGTGAGGA	GACTATGATA
1351	TAACATGTGT	ATGTAAATCT	TCATGTGATT	GTCATGTAAG	GCTTAACACA
1401	GTGGGTGGTG	AGTTCTGACT	AAAGGTTACC	TGTTGTCGTG	АААААААА
1451	AAAA (SEQ I	D NO: 2)		Fi	G. 11.